Prolongation of Intrasplenic Islet Xenograft Survival

CHRISTINE G. JANNEY, MD, PAUL E. LACY, MD, PhD, EDWARD H. FINKE, BS, and JOSEPH M. DAVIE, MD, PhD

Department of Pathology, Washington University Medical School, St. Louis, Missouri

The spleen has been examined as a possible site for transplantation of rat islets into diabetic mice. Marked prolongation of islet xenograft survival in the spleen can be achieved with *in vitro* culture (24 C) and a single injection of either rabbit antiserum to mouse lymphocytes (MALS) alone or MALS and rabbit antiserum to rat lymphocytes (RALS) into the recipients. The percentage of survival of intrasplenic xenografts at 100 days was 16%, as compared with 70% when rat islet xenografts were transplanted via the portal vein. Further improvement in pretreatment regimens will be needed before the spleen can be used as an effective site for possible future islet allograft or xenograft transplants in man. (Am J Pathol 1982, 107:001–005)

PROLONGATION of islet allograft survival without continuous immunosuppression has been accomplished in previous studies in our laboratory, using the combination of low temperature islet culture and a single injection of antilymphocyte serum (ALS).[1] Rejection of successful islet allografts was induced by administration of donor peritoneal exudate cells to the recipients.[2] Lafferty et al reported that marked prolongation of mouse thyroid allograft survival could be achieved with *in vitro* culture of donor thyroid for 26 days in an atmosphere of 95% O_2 and 5% CO_2.[3] It was suggested that culture had diminished or altered passenger leukocytes in the grafts, which appear to be necessary for initiation of graft rejection. Recently, successful islet xenografts were performed in our laboratory with the use of the low-temperature *in vitro* culture technique plus a single injection of ALS.[4,5] In these studies, 70% of the islet xenografts (rat to mouse) survived and maintained normoglycemia at 100 days after transplantation.

The site of transplantation of islet allografts and xenografts in our laboratory has been the liver, administered via the portal vein. The eventual application of islet allografts or xenografts to transplantation of islets into human diabetics requires investigation of other sites. The spleen is an attractive site for islet transplantation because of its high vascularity, portal venous drainage, and expendability should the grafts need to be recovered. Successful intrasplenic isografts and autografts have been reported,[6–10] although twice as many islets are required for reversal of the diabetic state and good control of glucose homeostasis.[7] Intrasplenic allografts have been reported to reject slightly faster but at a rate statistically comparable to the rejection rate of intraportal transplants.[8–10]

The purpose of this study was to determine whether we could successfully transplant rat islets into the spleens of diabetic mice, using low temperature culture of rat islets and a single injection of antilymphocyte serum at the time of transplantation.

Materials and Methods

Transplant Recipients

Male BALB/c mice were used as recipients, and, following at least three normal, nonfasting plasma glucose levels, were made diabetic by intravenous injection of streptozotocin (200 mg/kg of body weight). Only mice with nonfasting plasma glucose levels persistently >400 mg/dl were used as recipients. Mice were bled three times weekly before and after transplantation and were weighed daily. Graft rejection was considered to have occurred when posttransplant nonfasting plasma glucose levels exceeded 200 mg/dl.

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Address reprint requests to Paul E. Lacy, MD, Department of Pathology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110.

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Table 1—Survival of Intrasplenic Islet Xenografts

Group	Transplant survival* Individual days	Mean ± SEM
I. Control (Fresh islets)	3, 3, 4, 4, 5, 6, 6, 7, 8	5.1 ± 0.6
II. Fresh Islets MALS + RALS (IP)	8, 8, 8, 9, 10, 15†	9.7 ± 1.1
III. Cultured islets (24 C) MALS + RALS (IV)	14, 18, 24, 33, 39, >100‡	>38.0 ± 12.9
IV. Cultured islets (24 C) MALS + RALS (IP)	8, 13, 13, 14, 14, 32, 34, 38, 54, 64§	28.4 ± 6.1
V. Cultured islets (24 C) MALS (IP)	7, 12, 12, 34, 50, >100‖	>35.8 ± 14.5
VI. Cultured islets (24 C) RALS (IP)	7, 8, 9, 11, 11, 29, 52¶	18.3 ± 6.3

* Rejection was considered to have been initiated when the nonfasting glucose concentrations exceeded 200 mg/dl.

† Group II versus Group I, $P < 0.005$.

‡ Group III versus Group I, $P < 0.01$.

§ Group IV versus Group I, $P < 0.005$; Group IV versus Group III, insignificant.

‖ Group V versus Group I, $P < 0.025$; Group V versus Group III or Group IV, insignificant.

¶ Group VI versus Group I, $P < 0.05$; Group VI versus Group V, insignificant.

Islet Isolation

Islets were isolated from male Wistar-Furth rats by the collagenase technique[11] and were separated by centrifugation on a Ficoll gradient.[12] Islets were removed from the gradient, and only clean islets free of vascular and ductal tissue were hand-picked with a Pasteur pipette with the use of a dissecting microscope. These islets were examined under a microscope with reflected green light illumination, which allows small lymph nodes to be distinguished from islets and removed.[13]

Islet Culture

Isolated islets were maintained *in vitro* in CMRL-1066 tissue culture medium containing fetal calf serum (10%), D-glucose (150 mg/dl), penicillin (100 U/ml), and streptomycin sulfate (100 μg/ml). The islets were incubated in plastic culture dishes in an atmosphere of air and 5%CO_2 at 24 C for 7 days.

Transplants

Rat islets were injected directly into the splenic pulp of each diabetic mouse via a glass pipette. Initial studies indicated that islets injected directly into the spleen could embolize to the liver since islets could be found in histologic sections of the liver 1–4 days after transplantation. Thus the splenic vessels were clamped with a small bulldog clamp prior to injection of islets into the spleen in order to diminish the possibility of embolization to the liver.

Intrasplenic transplantation of 1000 rat islets, approximately double the number used in the xenografts transplanted via the portal vein,[4] produced normoglycemia within 2–5 days. Thus, 1000 fresh or cultured rat islets were transplanted into each diabetic recipient. Rabbit antiserum to rat lymphocytes (RALS) and mouse lymphocytes (MALS) were administered either intraperitoneally (IP) at the time of transplantation or intravenously (IV) 5–10 minutes prior to transplantation. The 6 experimental groups were as follows: transplants of freshly isolated islets, Group I without ALS, Group II with MALS (0.2 ml) and RALS (0.1 ml) given IP; transplants of rat islets cultured at 24 C for 7 days, Group III with both MALS (0.2 ml) and RALS (0.1 ml) given IV or IP (Group IV), Group V with MALS (0.3 ml) given IP, Group VI with RALS (0.1 ml) given IP.

Results

As shown in Table 1, transplants of freshly isolated rat islets (Group I) were rejected between 3–8 days, with a mean survival time (MST) of 5.1 ± 0.6 days. Administration of a single injection of MALS and RALS to the recipients receiving fresh rat islets (Group II) produced a slight but significant increase in MST to 9.7 ± 1.1 days ($P < 0.001$). The rate and pattern of rejection in all animals in these groups was acute, with plasma glucose levels rising rapidly from the time of rejection (defined as the day the nonfasting plasma glucose exceeds 200 mg/dl) to complete rejection 10–15 days later, when the plasma glucose returned to pretransplant levels (Figure 1).

In our previous study on obtaining marked prolongation of islet xenograft survival, the rat islets were maintained at 24 C for 7 days, and a single injection of both MALS (0.2 ml) and RALS (0.1 ml) was administered IV a few minutes prior to transplantation via the portal vein.[4,5] In the present investigation, this same pretreatment regimen (Group III) produced a marked prolongation of intrasplenic islet xenograft survival with MST of 38.0 ± 12.9 days (Table 1). One of the recipients was still normoglycemic at 100 days after transplantation.

In order to determine whether the route of administration of ALS was of importance in prolonging survival, another group of recipients received intrasplenic transplants of cultured rat islets, and the MALS and RALS was injected intraperitoneally (Group IV) instead of intravenously (Group III). A marked prolongation of islet xenograft survival was also obtained with the intraperitoneal route of administration of ALS with an MST of 28.4 ± 6.1 days. This survival time was not significantly

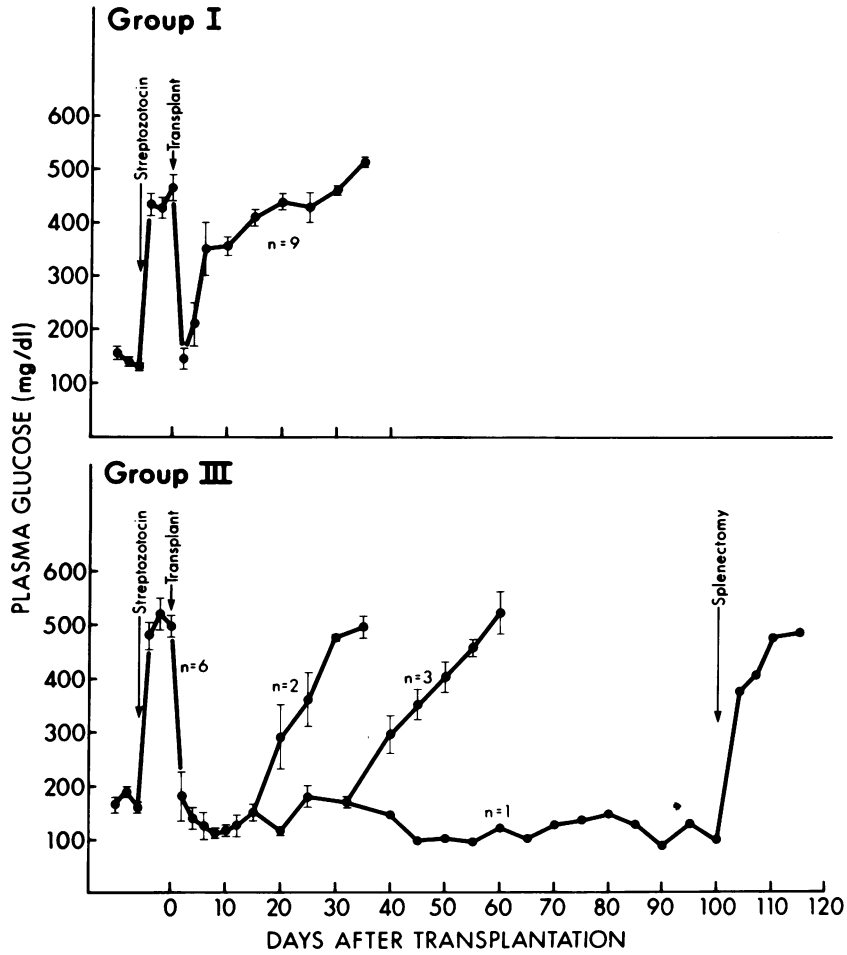

Figure 1—Nonfasting plasma glucose levels in diabetic mice following transplantation of fresh Wistar-Furth rat islets (Group I) and cultured rat islets (24 C) with one injection of MALS and RALS into the recipients at the time of transplantation (Group II). A splenectomy was performed on the 1 recipient that was still normoglycemic at 100 days after transplantation.

different from that of those receiving the antilymphocyte serum intravenously.

Studies were performed to determine whether RALS played a significant role in prolonging the survival of rat islet xenografts. Cultured rat islets were transplanted, and either 0.3 ml of MALS alone (Group V) or 0.1 ml of RALS alone (Group VI) was injected intraperitoneally following transplantation. Omission of RALS and the use of only MALS with low-temperature culture of the islets produced an MST of 35.8 ± 14.5 days. One recipient had not rejected the islets at 100 days after transplantation. This survival time was almost identical to that obtained when both MALS and RALS were used (Groups III and IV, Table 1). The use of RALS alone with low-temperature culture produced a lower MST (18.3 ± 6.3 days); however, the MST was not significantly different from that of the groups receiving MALS and RALS or MALS alone (Table 1).

A splenectomy was performed on the 2 recipients that were still normoglycemic at 100 days after transplantation. As shown in Figure 1, the plasma glucose levels in one of the recipients increased rapidly and reached the pretransplant diabetic level in 10 days. Histologic examination of the spleen revealed many islets, with no evidence of lymphocyte infiltration into the islets. The beta cells were intact, with a normal degree of beta granulation (Figure 2).

The plasma glucose levels in the other recipient did not increase following splenectomy. However, histologic examination of the spleen in this animal revealed islets with a normal degree of beta granulation. The failure to obtain a return to a diabetic state following splenectomy in this animal may have been due to the embolization of islets into the liver at the time of transplantation. Examination of histologic sections of the liver failed to reveal islets; however, they may have been missed due to the limited sampling of the liver tissue.

Discussion

The present study indicates that significant prolongation of islet xenograft survival can be achieved with intrasplenic transplants of rat islets into diabetic

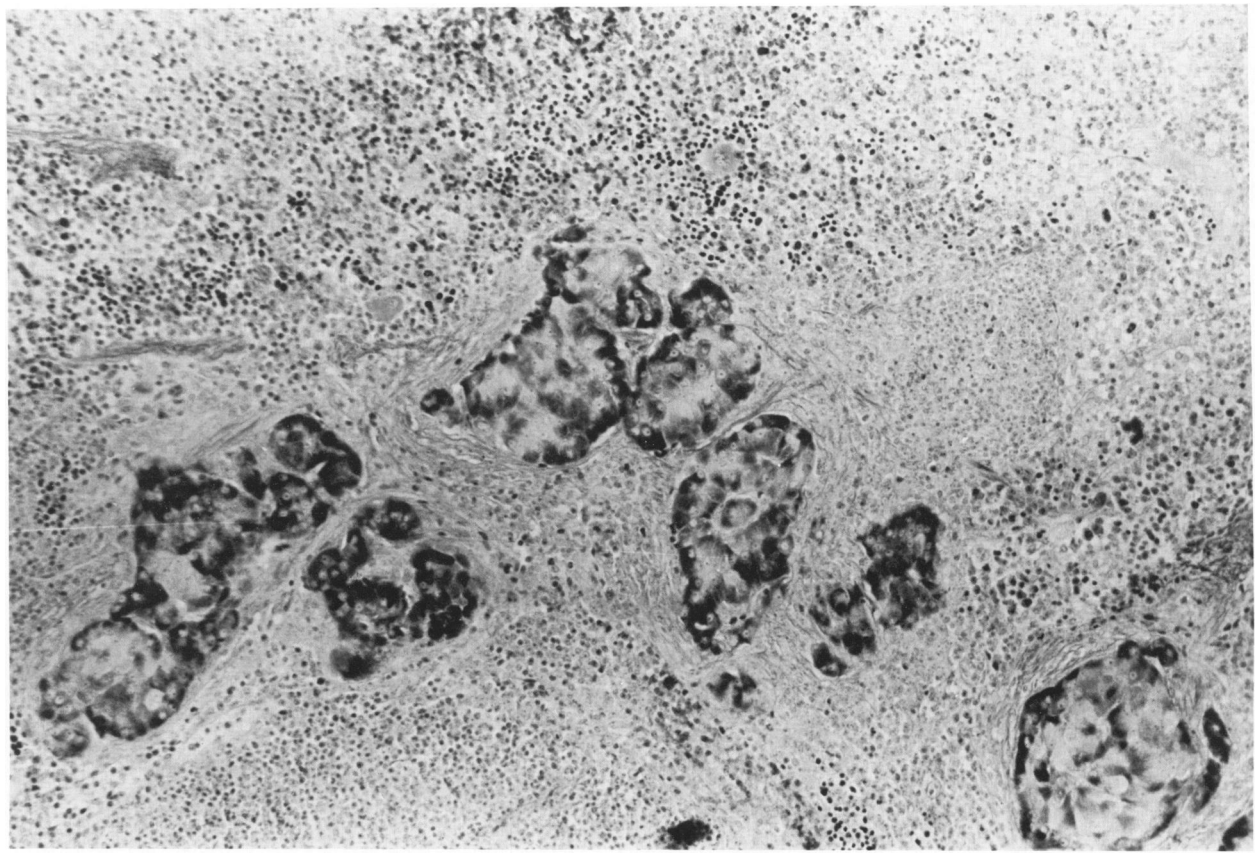

Figure 2— Photomicrograph of intact rat islets in the spleen of a recipient mouse at 100 days after transplantation. The rat islets were cultured (24 C) for 7 days prior to transplantation, and the recipient received one injection of MALS and RALS intravenously at the time of transplantation. The beta cells have a normal degree of granulation. (Aldehyde fuchsin stain)

mice by the use of *in vitro* culture of rat islets for 7 days at 24 C in conjunction with a single injection of either MALS and RALS or MALS alone into the recipients. The MST of the islet xenografts with MALS alone was 35.8 ± 14.5 days, and with both MALS and RALS the MST was 38.0 ± 12.9 days. One recipient in each of these groups was still normoglycemic at 100 days, and histologic examination of the spleen at this time revealed intact rat islets with a normal degree of beta granulation. One of these animals returned to a diabetic state following splenectomy at 100 days, whereas the other animal failed to become diabetic, even though islets were present in the spleen. It is possible that islets had embolized to the liver in this animal at the time of transplantation.

It is of interest that a single injection of MALS alone following transplantation of cultured rat islets produced a mean survival time comparable to that obtained when both MALS and RALS were used. In our previous studies[4,5] both MALS and RALS were required for marked prolongation of rat islet xenografts transplanted via the portal vein. In the groups receiving only MALS, the amount used in the present study was greater (0.3 ml) than that used in the previous study (0.2 ml). Thus it may be that the predominant effect of the single injection of antilymphocyte serum is on the immune system of the recipient. The use of RALS alone (Group VI) produced prolongation of survival with an MST that was much lower (18.3 ± 6.3 days) but statistically not significantly different from the group receiving MALS alone (35.8 ± 14.5 days).

Injecting the rat islets into the splenic parenchyma places the islets in immediate and direct contact with the lymphoid system of the mouse. Theoretically, this could create a favorable environment for nonspecific destruction of islet cells as well as initiating immune destruction with a very low level of contamination of the grafts by passenger lymphoid cells. Thus it is most encouraging that a marked prolongation of islet xenograft survival could be obtained with this site of transplantation.

With the present state of technology for prevention of immune rejection of the islets, the liver appears to be a more favorable site for islet xenograft transplants than the spleen. In our previous studies[4,5] on

transplanting rat islet xenografts via the portal vein into diabetic mice, 7 of 10 recipients were still normoglycemic at 100 days, whereas only 2 of 12 recipients were normoglycemic at 100 days in the present investigation. These findings do not eliminate consideration of the spleen as the site for possible future islet allograft or xenograft transplants in man; however, further improvements will be needed in the methods used for prevention of immune rejection by pretreatment regimens before this site could be used effectively in man.

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